Small Pox Specimen Information for Utah Department of Health Laboratory
Please contact Dr. Pounder (801) 584-8449 prior to submitting the specimen for testing.

The Utah Department of Health Laboratory can test for the presence of Variola virus, Vaccinia virus, and Varicella Zoster virus at this time. Outlined below are guidelines for specimen collection and transport. Please handle all potential specimens with care.

Specimen Handling

A. Specimen Collection-Safety

1. Only personnel who have been successfully vaccinated against smallpox in the last 3 years or non-vaccinated personnel with no contraindication to vaccination should participate in specimen collection. Vaccination of non-vaccinated personnel should occur as soon as possible after specimen acquisition.

2. All procedures for obtaining, processing, packing, and shipping potentially infectious material should be performed by using BSL-2, or if available, BSL-3 practices. Appropriate respiratory and barrier protective equipment such as N-95 or HEPA-filtered mask, gloves, gown, shoe covers, and protective eyewear should be used in specimen collection for suspected cases of smallpox.

3. While working with specimens, laboratory personnel should avoid any activity that brings hands or fingers in contact with mucosal surfaces, such as eating, drinking, smoking, adjusting contacts, or applying makeup.

4. After removing gloves, personnel should thoroughly wash their hands with antimicrobial soaps before leaving the laboratory. Areas of skin known or suspected to have come in contact with variola virus should be washed with the recommended soap, followed by 60-95% ethanol containing gel (available from Fisher Scientific (800) 766-7000). If possible, skin should be decontaminated with 0.5% sodium hypochlorite for at least one minute.

B. Specimen Collection

Collection Kit Guidelines

Test Request form available soon at www.health.utah.gov/els/microbiology under the Bioterrorism heading

Personal Protective Equipment: gloves, gown, shoe covers, N95 mask, and eye protection
Alcohol wipes
Scalpel with a number 10 blade
26 gauge needle
Wooden applicator
1.5 to 2 ml screw capped plastic sample tubes
snap capped plastic sample tubes
formalin for fixed tissues (Sigma (800) 325-3010))
clean glass microscope slides
50 ml plastic conical centrifuge tubes for holding glass slides or commercial slide holders
Polyester swabs
Permanent marking pens for labeling tubes
Tiger-top vacutainer tube for serum collection 10cc
Purple-top vacutainer tube for whole blood collection 5cc

Vacutainer collection supplies
Sharps containers
M4 transport medium (REMEL (800)255-6730)
Gauze
Most items are available from Fisher Scientific (800)766-7000, VWR (800)932-5000 or ISC BioExpress (800)999-2901
Disclaimer: Names of vendors or manufacturers are provided as examples of suitable product sources; inclusion does not imply endorsement by the Utah Department of Health.

1. Label all tubes, vials, and microscope slide holders with patient's name, unique identifier, date of collection, source of specimen (vesicle, pustule, scab, or fluid), and name of person collecting sample.

2. Avoid cross-contamination of samples. Use one sample per primary container. Collect sufficient amount of lesion material to permit multiple diagnostic tests and confirmations. Suitable specimens for virologic tests of suspected smallpox cases are biopsy tissues, 2 to 4 scabs, and vesicular tissues and fluid. Serologic testing requires at least 1 ml of serum. Other potentially useful specimens for viral sampling are throat swabs and whole blood, however, quality-controlled diagnostic tests have not been completed for these specimens.

3. Biopsy specimens of individual lesions should be made with a 3.5 or 4.5 mm punch biopsy device. This should sample the entire lesion. The biopsied material should be bisected with sterile scissors or scalpel and placed into 2 labeled containers. One half of the sample should be placed in formalin for immunohistochemical or histopathological evaluation, this container should be kept at room temperature. The other half of the lesion material should be placed in a dry, sterile 1.5 to 2 ml screw capped sample tube, do not add transport medium. Refrigerate these containers if shipment occurs within 24 hours. If shipment will be longer than 1 day, freeze this sample.

4. Scabs should be removed by using a sterile 26 gauge needle and collected in dry, sterile 1.5 to 2 ml screw capped sample tube.

5. Vesicular material should be sampled after the skin area has been sanitized with an alcohol wipe and allowed to dry. Unroof the lesion with a sterile 26 gauge needle or with a scalpel. Place the skin "roof" in a dry, sterile 1.5 to 2 ml screw capped sample tube. Scrape the base of the blister with a wooden applicator and smear the scrapings onto a clean glass light-microscope slide. Touch a clean glass light-microscope slide to the open lesion multiple times. If additional tests are required electron microscope slides will be provided. Lightly touch the shiny side of 1 or 2 plastic coated grids to the base of the open lesion. Allow slides and grids to air dry for approximately 5 minutes, then place in appropriate containers. Repeat this procedure for 2 or more lesions.

6. Autopsy specimens from major organs collected for virus isolation and immunohistochemical and histopathological evaluation include skin, spleen, lymph node, liver, lung, kidney, and heart. Specimens for virus isolation should be frozen. Specimens for immunohistochemical and histopathological evaluation should be fixed in formalin.

7. Blood should be drawn into a purple-topped tube for possible viral identification and into a marble-topped serum separator tube for serological testing.

8. A cotton or polyester swab should be used for sampling tonsillar tissue in the posterior pharynx. Collect the specimen as a throat swab. Break off the end of applicator into a 1.5 to 2 ml screw capped sample tube. Do not add transport medium.

9. Package each patient's lesion specimens separately to avoid cross-contamination. See the next section for sample transport information.

10. When specimen collection has been completed, all protective materials and sample collection materials must be double bagged in biohazard bags and autoclaved or incinerated.

11. Specimens for Variola virus testing are dried vesicular fluid on a microscope slide, vesicular tissue (skin from unroofed vesicle), swab of vesicular fluid. Specimens for Variola virus PCR should be dried vesicular fluid on slide, scab or swab material. To obtain a microscope slide preparation, unroof the scab with a sterile scalpel or a 26 gauge needle. Place the scab in a labeled plastic sample tube, do not add transport medium. Touch a clean glass microscope slide to the open lesion several times. Allow the slide to air dry and place in an appropriate labeled container. Swabs of vesicular fluid should be collected by vigorously scrubbing the base of an unroofed lesion with a sterile swab. A polyester swab is preferred. Contamination with blood is not a concern for this test. Place swab in a snap cap tube or other suitable container. Break off the stick if necessary. Do not add transport fluid. Specimens for PCR testing, may be stored indefinitely and shipped at room temperature.

12. Specimens from cases that require rule-out testing for Varicella Zoster Virus (VZV) will be analyzed by the direct fluorescent antibody (DFA) method. To collect specimens for the DFA test, unroof the vesicle using a 26 gauge needle. Scrub the base of the lesion vigorously enough with a sterile swab to collect lesion cells. Avoid contaminating the sample with blood. Place the swab in a sterile tube containing 1 to 2 ml of suitable transport medium (M4 viral transport medium) Keep swab moistened. Transport with a cold pack, do not freeze. Specimens for VZV PCR should be scab or swab material. Collect by lifting the scab using the beveled point of a sterile 26 gauge needle and place in a snap cap tube or other suitable container. Do not add transport medium. Swabs of vesicular fluid should be collected by vigorously scrubbing the base of an unroofed lesion with a sterile swab. A polyester swab is preferred. Contamination with blood is not a concern for this test. Place swab in a snap cap tube or other suitable container. Break off the stick if necessary. Do not add transport fluid. Specimens for PCR testing, may be stored indefinitely and shipped at room temperature.

13. Specimens that require testing to rule-out Vaccinia virus will be analyzed by PCR. To obtain a microscope slide preparation, unroof the scab with a sterile scalpel or a 26 gauge needle. Place the scab in a labeled plastic sample tube. Touch a clean glass microscope slide to the open lesion several times. Allow the slide to air dry and place in an appropriate labeled container.

C. Specimen Handling-Shipping

1. Specimens should be packed using the "triple" packaging scheme of primary receptacle, water-tight secondary packaging and durable outer packaging. Adequate absorbent material will be packed with the specimens to contain all fluids. A rigid, crush-proof overpack should be used. No transport medium or glycerol will be added to the specimens. Formalin-fixed, electron microscopy grids, and PCR specimens should be stored and shipped at room temperature, not frozen. These specimens should not be packed with dry ice as dry ice vapors may cause a change in the pH of the specimens. Additional specimens should be stored at 2-8˚C or frozen according to specific directions for the type of specimen. All IATA regulations should be adhered to for preparing packages, labeling packages, and paperwork to accompany the specimens. Transport by courier should follow established safety guidelines.